Taiwanese Journal of Obstetrics & Gynecology 54 (2015) 588–591

Contents lists available at ScienceDirect

Taiwanese Journal of Obstetrics & Gynecology

journal homepage: www.tjog-online.com

Original Article

Interphase fluorescence *in situ* hybridization assisting in prenatal counseling for amniocentesis karyotyping-detected fetal mosaicism

Sheng-Yuan Su [a], Ho-Yen Chueh [a], Ching-Pei Li [b], Yao-Lung Chang [a], Shuenn-Dyh Chang [a, *], Chih-Ping Chen [c, d, e, f, g, h]

[a] *Department of Obstetrics and Gynecology, Chang Gung Memorial Hospital, Lin-Kou Medical Center, Chang Gung University, Taoyuan, Taiwan*
[b] *Cytogenetic Laboratory, Linkou Chang Gung Memorial Hospital, Taoyuan, Taiwan*
[c] *Department of Obstetrics and Gynecology, Mackay Memorial Hospital, Taipei, Taiwan*
[d] *Department of Medical Research, Mackay Memorial Hospital, Taipei, Taiwan*
[e] *Department of Biotechnology, Asia University, Taichung, Taiwan*
[f] *School of Chinese Medicine, College of Chinese Medicine, China Medical University, Taichung, Taiwan*
[g] *Institute of Clinical and Community Health Nursing, National Yang-Ming University, Taipei, Taiwan*
[h] *Department of Obstetrics and Gynecology, School of Medicine, National Yang-Ming University, Taipei, Taiwan*

ARTICLE INFO

Article history:
Accepted 8 July 2015

Keywords:
counseling
interphase fluorescence *in situ* hybridization
karyotype
mosaicism

ABSTRACT

Objective: To evaluate how interphase fluorescence *in situ* hybridization (FISH) played a role in genetic counseling when encountering prenatally detected fetal mosaicism cases.
Materials and methods: We retrospectively reviewed 17 cases of amniotic fluid specimens diagnosed with Level III chromosome mosaicism using *in situ* coverslip culture method. Among them, seven received additional interphase FISH tests; five were related to autosomal mosaicism and two others were due to sex chromosomes.
Results: In the autosome group, one couple chose to terminate the pregnancy due to a high percentage of trisomy 21 cells (48.1%) shown on interphase FISH; in the gonosome group, one case chose termination as FISH exhibited as high as 80% of XXYY cells.
Conclusion: Performing interphase FISH on uncultured amniocytes for cases detected with mosaicism by traditional amniotic fluid culture provided quick confirmation of the karyotyping results; additionally, obtaining information about the extent of the abnormality involved using interphase FISH could also play a role in counseling patients on the decision making concerning the future of their pregnancies.

Introduction

Fluorescence *in situ* hybridization (FISH) was first introduced as a potentially powerful tool in clinical cytogenetics. Interphase FISH with alpha satellite probes or locus-specific probes can be used for rapid prenatal diagnoses of numerical or structural chromosomal anomalies from direct culture of amniocytes [1]. Because the amniotic fluid is composed of cells from all three germ layers of the fetus (i.e., endoderm, mesoderm, and ectoderm), cytogenetic culture result from amniocentesis is more representative of the fetus as a whole compared with chorionic villous sampling or cordocentesis, the former being extra-embryonic and the latter representing only the mesoderm. In the *in situ* coverslip culture method for amniotic fluid specimens, when different cytogenetic findings are detected in more than one colony of cells, fetal mosaicism is indicated [2]. In the absence of detectable fetal anomalies by ultrasound or other imaging modalities, parents expecting the mosaicism results from amniocentesis can be experiencing a tremendous amount of anxiety and stress, not knowing what to do with the future of their pregnancy. Interphase FISH with appropriate probes on uncultured amniocytes can better reflect the actual state of the fetus rather than traditional chromosome analysis, which uses cultured cells. Interphase FISH results coupled with proper genetic counseling can assist the parents facing the dilemma of fetal mosaicism in making an educated choice for the future of their pregnancies.

* Corresponding author. Department of Obstetrics and Gynecology, Chang Gung Memorial Hospital, 5, Fu-Hsing Street, Taoyuan, Taiwan.
E-mail address: gene@cgmh.org.tw (S.-D. Chang).

http://dx.doi.org/10.1016/j.tjog.2015.08.019

Materials and methods

This is an Institutional Review Board-approved retrospective study. From January 2010 to June 2014, genetic amniocentesis results from the cytogenetic laboratory of Linkou Chang Gung Memorial Hospital (Taoyuan, Taiwan) were reviewed and those with mosaicism findings singled out.

Conventional cytogenetic analysis

Approximately 15–20 mL of amniotic fluid was set up for *in situ* coverslip culture following standard cytogenetic protocol. Chromosome analysis using G-banding techniques at 550 bands of resolution was performed on each colony of cells. Between 15 and 20 colonies were examined and karyotypes were produced. A diagnosis of fetal mosaicism was made if at least two colonies of cells showed different chromosomal constitution [3].

Fluorescence in situ hybridization

Interphase FISH procedure was performed, basically following a standard protocol proposed by Klinger et al [1] but with some modifications. The procedure involved the following steps: (1) the first step is the *sample preparation step* in which uncultured amniotic fluid cells were collected by centrifugation, and then concentrated down the center of the slide to obtain 300–400 cells/slide, followed by hypotonic solution (0.075M KCl) and fixative (methanol:acetic acid; 3:1) treatment; (2) the second step is *denaturation* that involved simultaneous denaturation (at 75°C for 8 minutes) of probe and target DNA under a sealed coverslip; (3) the third step is *hybridization* in which a hybridization mixture placed on the slide was incubated overnight in a moist chamber at 37°C; (4) in the fourth step, the slides were washed in a postwash solution (*postwashing*) of 50% formamide and 2× saline-sodium citrate; (5) the fifth step is *detection/staining*, in which the slides were stringently washed and counterstained with 4′,6-diamidino-2-phenylindole/antifade reagent, and then placed under a fluorescence microscope using a dual band-pass filter to visualize fluorescein isothiocyanate and Texas red simultaneously; and (6) the sixth and final step is *analysis* in which a minimum of 40 hybridized nuclei (range 40–100) were counted for each sample, and the number of nuclei exhibiting one, two, three, and four hybridization signals were recorded.

The following were the related FISH probes applied in different mosaicism cases:

1. For trisomy 20 mosaicism, bacterial artificial chromosome (BAC) clone probe RP11-2E8 (20p12.2; green spectrum) was used.
2. For iso20q mosaicism, a 20-q-specific probe RP11-266K16 (20q13.33) in the red spectrum and a 20-p-specific probe RP11-530N10 (20p13) in the green spectrum were used.
3. For mosaic ring marker 8, an 8p11.1-q11.2-specific probe (Vysis CEP8,D8Z2; spectrum green) was used.

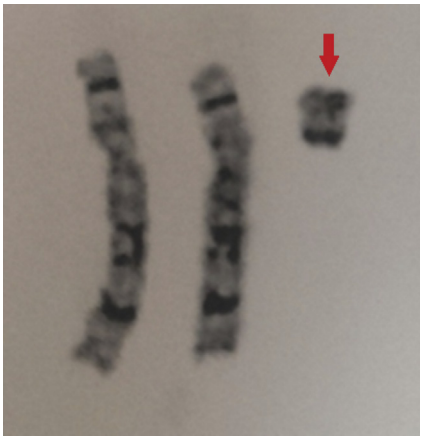

Figure 1. A partial G-banded karyotype. The arrow indicates the supernumerary ring chromosome 8 marker.

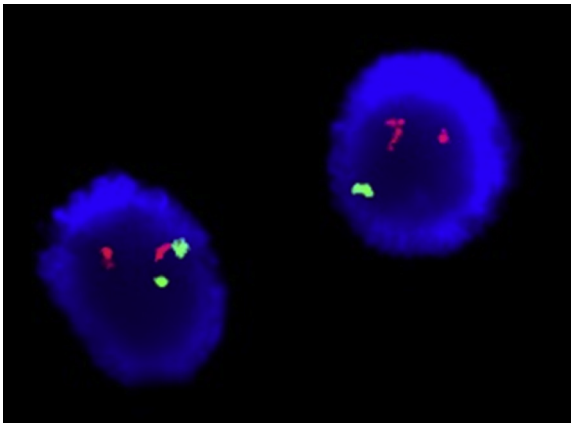

Figure 2. Interphase fluorescence *in situ* hybridization analysis on uncultured amniocytes using CEPX (spectrum green) and CEPY (spectrum red) probes, showing an XXYY on the left-side cell and an XYY on the right-side cell.

4. For mosaic trisomy 21, a BAC clone probe RP11-91N21 (21q11.2; spectrum green) was used.
5. For mosaic trisomy 12, a 12q11-q12-specific probe RP11-496H24 (spectrum green) was used.
6. For the other two cases with sex chromosomes mosaicism, the CEPX (DXZ1, Vysis) probe corresponding to Xp11.1-q11.1 labeled with spectrum green, and the CEPY (DYZ3, Vysis) probe corresponding to Yp11.1-q11.1 labeled with spectrum orange were used.

Results

A total of 17 true mosaicism (or Level III) cases were detected of the 6436 amniocenteses cases, resulting in a 0.26% incidence of

Table 1
Mosaicism cases receiving additional interphase FISH tests.

Conventional cytogenetic analysis	Percentage of abnormal cells	FISH	Phenotype	Outcome
47,XY,+mar[2]/46,XY[19] (*marker*: ring chromosome 8)	2/21, 9.5%	8/40, 20.0%	Bilateral pyelectasis	Continuing
46,XY,i(20)(q10)[5]/46,XY[21]	5/26, 19.2%	0/50, 0.0%	No obvious finding	Continuing
47,XY,+20[26]/46,XY[9]	26/35, 74.3%	5/50, 10.0%	No obvious finding	Continuing
47,XX,+21[6]/46,XX[14]	6/20, 30.0%	25/52, 48.1%	No obvious finding	Termination
47,XX,+12[8]/46,XX[12]	8/20, 40.0%	16/65, 24.6%	Large for gestational age	Continuing
48,XXYY[17]/47,XYY[3]	17/20, 85.0%	80/100, 80%	Bilateral renal enlargement	Termination
45,X[5]/46,XX[15]	5/20, 25.0%	23/100, 23%	No obvious finding	Continuing

FISH = fluorescence *in situ* hybridization.

Table 2
Mosaicism cases where FISH was not offered as a complementary test.

Conventional cytogenetic analysis	Phenotype	Outcome
46,XX,r(21[12]/45,XX,-21[5]	Sacrococcygeal teratoma	Termination
46,XX,der(11),t(11;?)(q23;?)[5]/46,XX[15]	Intrauterine growth restriction and congenital heart disease	Termination
47,XY,+21,+21,der(21;21)(q10;q10)[21]/46,XY[15]	Multiple anomalies	Termination
45,X[13]/47,XYY[5]/46,XY[6]	No obvious finding	Continuing
45,X[2]/46,XY[10]	No obvious finding	Continuing
45,X[19]/46,X,idic(Y)(p11.2)[9]	No obvious finding	Termination
47,XYY[2]/46,XY[18]	No obvious finding	Continuing
45,X[5]/47,XXX[14]	No obvious finding	Termination
45,X[10]/46,XX[10]	No obvious finding	Continuing
47,XXY[8]/46,XY[20]	No obvious finding	Continuing

FISH = fluorescence *in situ* hybridization.

chromosomal mosaicism. Among them, seven received additional interphase FISH tests, and the related cytogenetic findings, prenatal phenotypes, FISH results, and perinatal outcomes are listed in Table 1. For the two cases related to chromosome 20, trisomy 20, and isochromosome 20 q, FISH on uncultured amniocytes showed a much lower abnormality level (10% and 0%, respectively) than on the culture cells, and the pregnancy was allowed to proceed. For the three cases that exhibited mosaicism for a supernumerary marker (Figure 1; this case was later confirmed to be a ring chromosome 8, 8p11.1-q11.2), trisomy 12, and trisomy 45,X, the abnormality levels were low (approximately 20%) on uncultured cells, and thus the pregnancies were allowed to continue; however, in the other two cases, mosaic trisomy 21 and 48,XXYY (Figure 2), we found a high percentage of abnormal cells using interphase FISH (48% and 80%, respectively), and thus, the pregnancies were aborted. In terms of prenatal phenotypes, the mosaic ring 8 marker case showed bilateral mild fetal pyelectasis, which was resolved spontaneously at postnatal follow up; the mosaic trisomy 12 fetus was large for gestational age, not diabetes related, weighing 4300 g at delivery at 36 weeks of gestation from preterm premature of membrane; and the 48,XXYY mosaicism fetus exhibited bilateral renal enlargement on prenatal ultrasound. Overall, two of the seven cases opted for mid-trimester termination of pregnancy.

Table 2 lists the other 10 mosaicism cases for which FISH was not offered as a complementary test. Three marked phenotypes had been spotted prenatally, including one with a sacrococcygeal teratoma in a mosaic ring 21 fetus. Overall, five of the 10 cases did not continue the pregnancy.

Discussion

The incidence of true fetal mosaicism in our cytogenetic laboratory was 2.6/1000, a figure in line with a literature report, which revealed Level III chromosome mosaicism in approximately 2/1000 amniotic fluids [4]. Parents facing this problem had a dilemma as to whether to continue or abort the pregnancy. Parents whose fetuses are having severe abnormalities may have less trouble making the choice. Whereas parents of fetuses with mild prenatal phenotypes or nothing at all go through a difficult time while receiving genetic counseling or even waiting for the results of other complementary tests. Our experience indicates that interphase FISH results play an important role in decision making. Following the publication of several large studies documenting the accuracy of interphase FISH studies on uncultured amniotic fluid [5,6] and because of the short turnaround time, many tests are done to not only quickly confirm the traditional karyotype findings, but also to evaluate the extent of mosaic chromosomal abnormality by looking at the uncultured cells.

In our series, two cases related to chromosome 20 opted to continue the pregnancy: For the mosaic isochromosome 20q case, interphase FISH found no abnormal cells in contrast to 19% found on karyotyping with cultured cells. There had even been reports demonstrating cytogenetic discrepancies between cultured and uncultured amniocytes on this mosaicism [7]; in the mosaic 20 trisomy case, only 10% of abnormal cells were found on FISH against the 74% of trisomy 20 on cultured cells; Robinson et al [8] found abnormal outcome in 50% of pregnancies with 80% of trisomy 20 cells. Because of a favorable interphase FISH finding plus no significant phenotype detected by prenatal ultrasound, the couple opted to continue the pregnancy. For the other three cases that did not receive midtrimester termination, including supernumerary ring 8 marker, trisomy 12, and trisomy 45,X, the cultured and uncultured cells showed a comparably low level of abnormality and the phenotypes were all minor ones. Although the mosaic trisomy 12 case showed overgrowth on delivery (97th percentile), the follow-up visits found gradual normalization. Implications of increased dosage of chromosome 12 in relation to congenital overgrowth have already been reported [9]. Regarding the fetus with a mosaic ring 8 marker, the prenatal pyelectasis findings were not found on postnatal check-up. Renal abnormalities had been observed in patients with an additional ring marker 8. Spinner et al [10] documented a case with 56% mosaicism for a supernumerary marker 8p11-q11 in blood cells, who presented with mild hydronephrosis and kidney malrotation. In the last two cases that chose termination, abnormal levels of trisomy 21 and 48,XXYY were very high in both cultured and uncultured cells. For mosaic trisomy 21, there was a significant positive correlation between the percentage of mosaicism and the severity of phenotype [11]. With regard to the high level of 48,XXYY mosaicism, Linden et al [12] reviewed the phenotypes of sex chromosome polysomy with more than four gonosomes and concluded that in each, intellectual compromise or frank mental retardation was unavoidable [12].

In conclusion, we have demonstrated how interphase FISH was useful as a complementary test when counseling parents for fetal mosaicism detected by conventional cytogenetic analysis.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

References

[1] Klinger K, Landes G, Shook D, Harvey R, Lopez L, Locke P. Rapid detection of chromosome aneuploidies in uncultured amniocytes by using fluorescence *in situ* hybridization (FISH). Am J Hum Genet 1992;51:55–65.
[2] Cheung SW, Spitznagel E, Featherstone T, Crane JP. Exclusion of chromosomal mosaicism in amniotic fluid cultures: efficacy of *in situ* versus flask techniques. Prenat Diagn 1990;10:41–57.
[3] Featherstone T, Cheung SW, Spitznagel E, Peakman D. Exclusion of chromosomal mosaicism in amniotic fluid cultures: determination of number of colonies needed for accurate analysis. Prenat Diagn 1994;14:1009–17.
[4] Wilson MG, Lin MS, Fujimoto A, Herbert W, Kaplan FM. Chromosome mosaicism in 6,000 amniocenteses. Am J Med Genet 1989;32:506–13.

[5] Bryndorf T, Christensen B, Vad M, Parner J, Brocks V, Philip J. Prenatal detection of chromosome aneuploidies by fluorescence *in situ* hybridization: experience with 2000 uncultured amniotic fluid samples in a prospective preclinical trial. Prenat Diagn 1997;17:333–41.
[6] D'Alton ME, Malone FD, Chelmow D, Ward BE, Bianchi DW. Defining the role of fluorescence *in situ* hybridization on uncultured amniocytes for prenatal diagnosis of aneuploidies. Am J Obstet Gynecol 1997;176:769–74.
[7] Chen CP, Chern SR, Wu PS, Su JW, Chen YT, Chen LF, et al. Application of interphase fluorescence *in situ* hybridization to uncultured amniocytes for differential diagnosis of pseudomosaicism from true mosaicism in mosaic isochromosome 20q detected at amniocentesis. Taiwan J Obstet Gynecol 2013;52:450–3.
[8] Robinson WP, McGillivray B, Lewis ME, Arbour L, Barrett I, Kalousek DK. Prenatally detected trisomy 20 mosaicism. Prenat Diagn 2005;25:239–44.
[9] Chiurazzi P, Bajer J, Tabolacci E, Pomponi MG, Lecce R, Zollino M, et al. Assisted reproductive technology and congenital overgrowth: some speculations on a case of Pallister-Killian syndrome. Am J Med Genet A 2004;130A:315–6.
[10] Spinner NB, Grace KR, Owens NL, Sovinsky L, Pellegrino JE, McDonald-McGinn D, et al. Mosaicism for a chromosome 8-derived minute marker chromosome in a patient with manifestations of trisomy 8 mosaicism. Am J Med Genet 1995;56:22–4.
[11] Papavassiliou P, York TP, Gursoy N, Hill G, Nicely LV, Sundaram U, et al. The phenotype of persons having mosaicism for trisomy 21/Down syndrome reflects the percentage of trisomic cells present in different tissues. Am J Med Genet A 2009;149A:573–83.
[12] Linden MC, Bender BG, Robinson A. Sex chromosome tetrasomy and pentasomy. Pediatrics 1995;96:672–82.